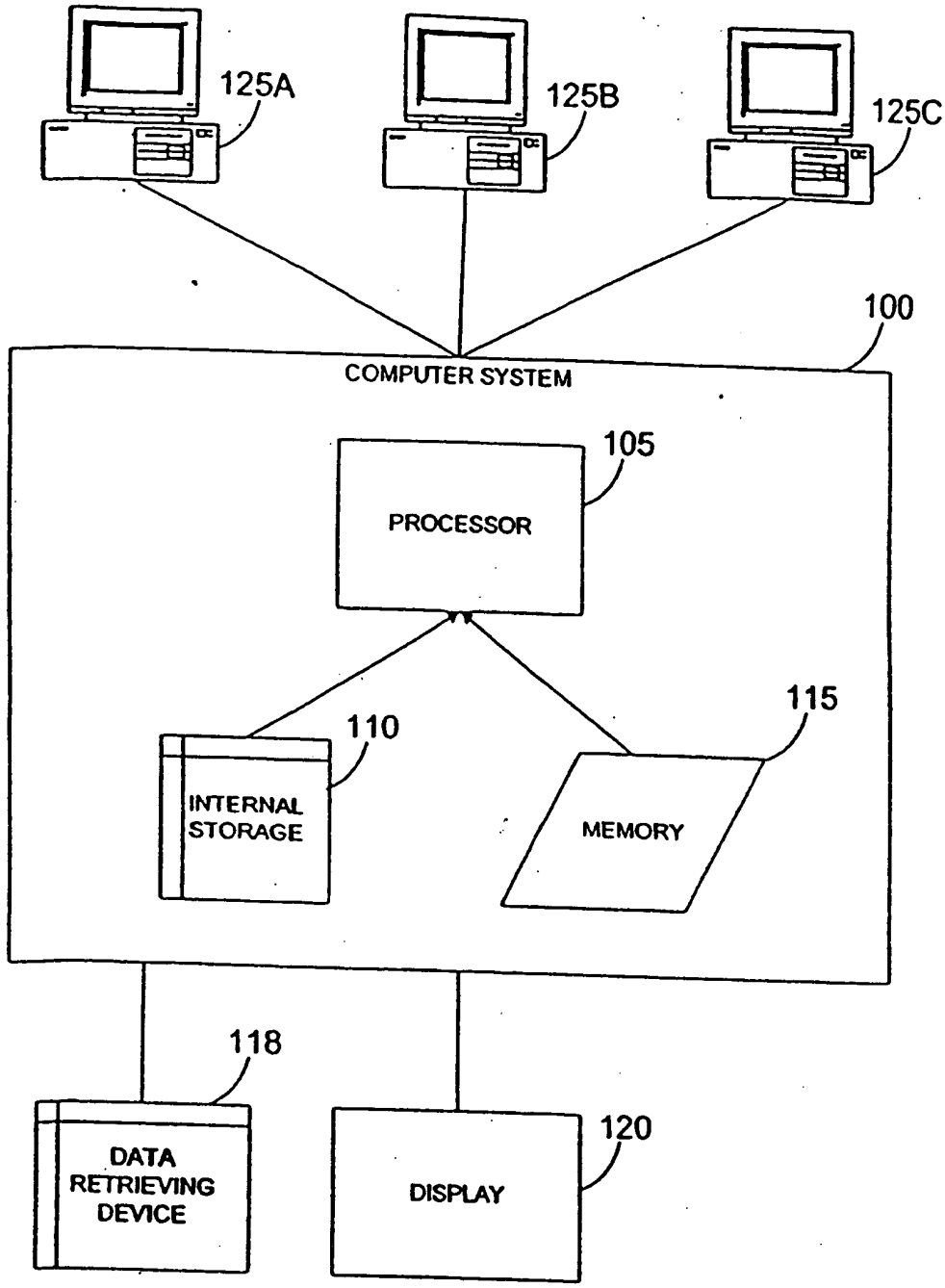

125A
125B
125C
100
COMPUTER SYSTEM
105
PROCESSOR
110
INTERNAL STORAGE
115
MEMORY
118
DATA RETRIEVING DEVICE
120
DISPLAY

FIGURE 1

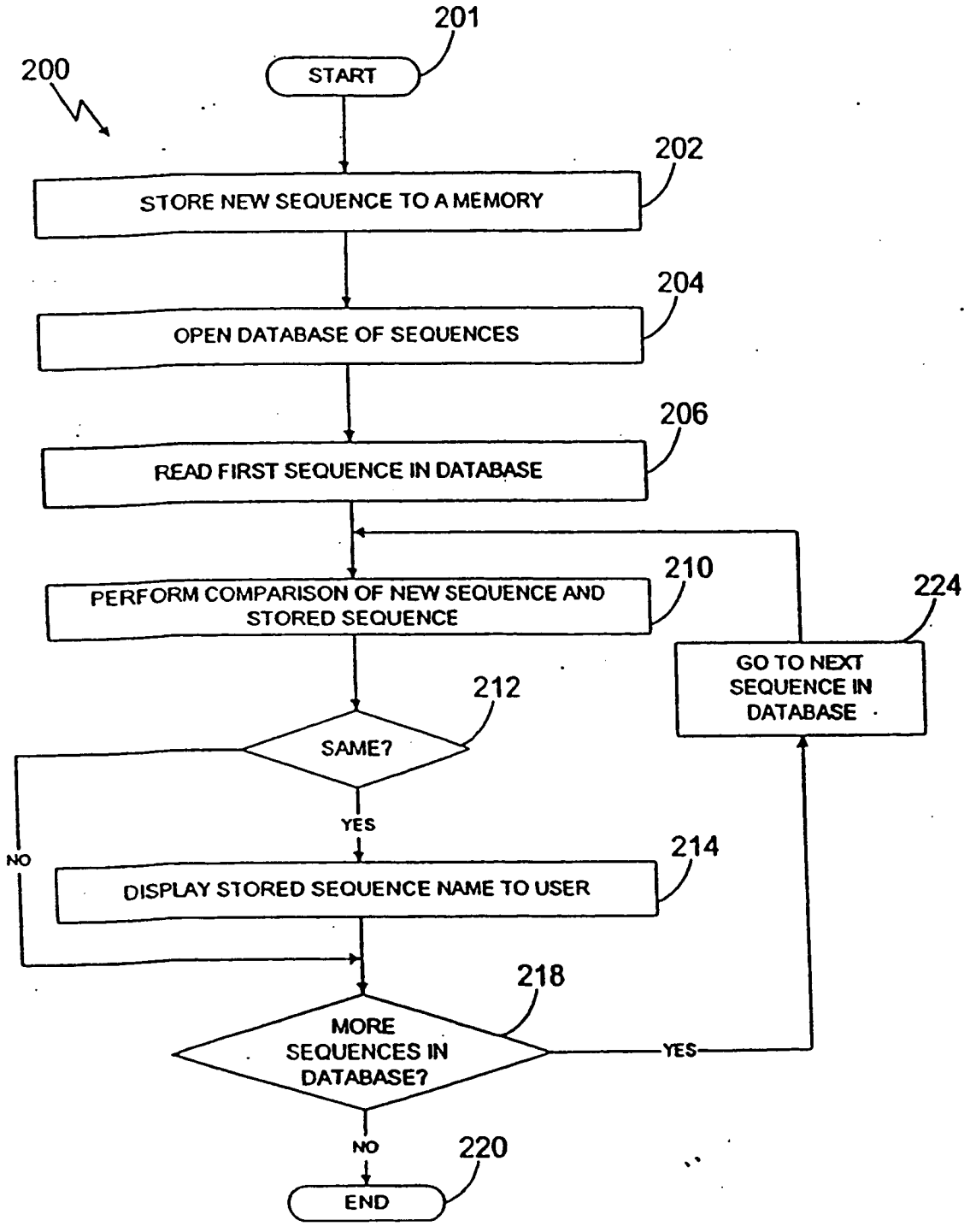

200
201
START
202
STORE NEW SEQUENCE TO A MEMORY
204
OPEN DATABASE OF SEQUENCES
206
READ FIRST SEQUENCE IN DATABASE
210
PERFORM COMPARISON OF NEW SEQUENCE AND STORED SEQUENCE
212
SAME?
YES
NO
214
DISPLAY STORED SEQUENCE NAME TO USER
218
MORE SEQUENCES IN DATABASE?
YES
NO
220
END
224
GO TO NEXT SEQUENCE IN DATABASE

FIGURE 2

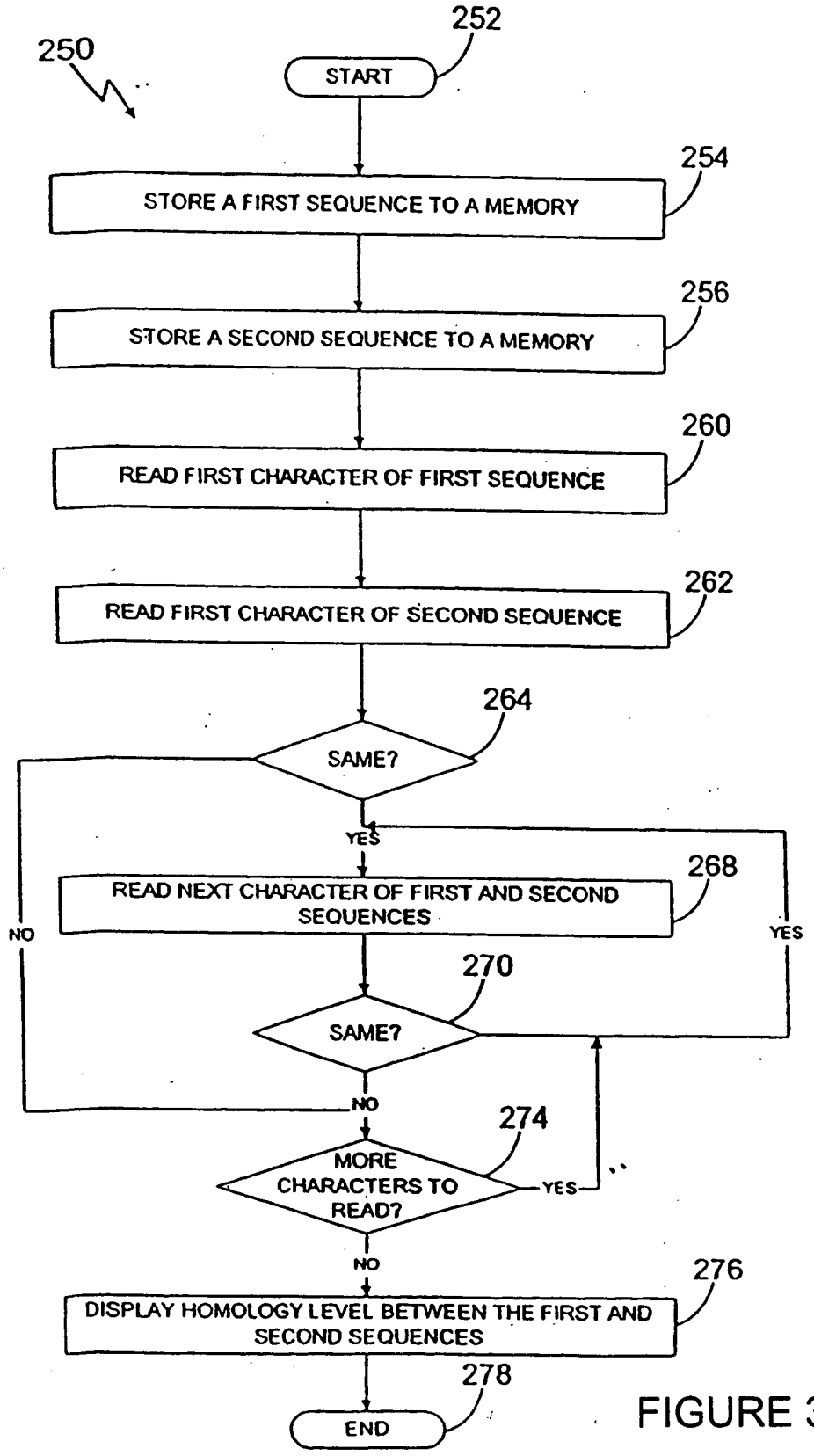

250
252
START
254
STORE A FIRST SEQUENCE TO A MEMORY
256
STORE A SECOND SEQUENCE TO A MEMORY
260
READ FIRST CHARACTER OF FIRST SEQUENCE
262
READ FIRST CHARACTER OF SECOND SEQUENCE
264
SAME?
YES
NO
268
READ NEXT CHARACTER OF FIRST AND SECOND SEQUENCES
YES
270
SAME?
NO
274
MORE CHARACTERS TO READ?
YES
NO
276
DISPLAY HOMOLOGY LEVEL BETWEEN THE FIRST AND SECOND SEQUENCES
278
END

FIGURE 3

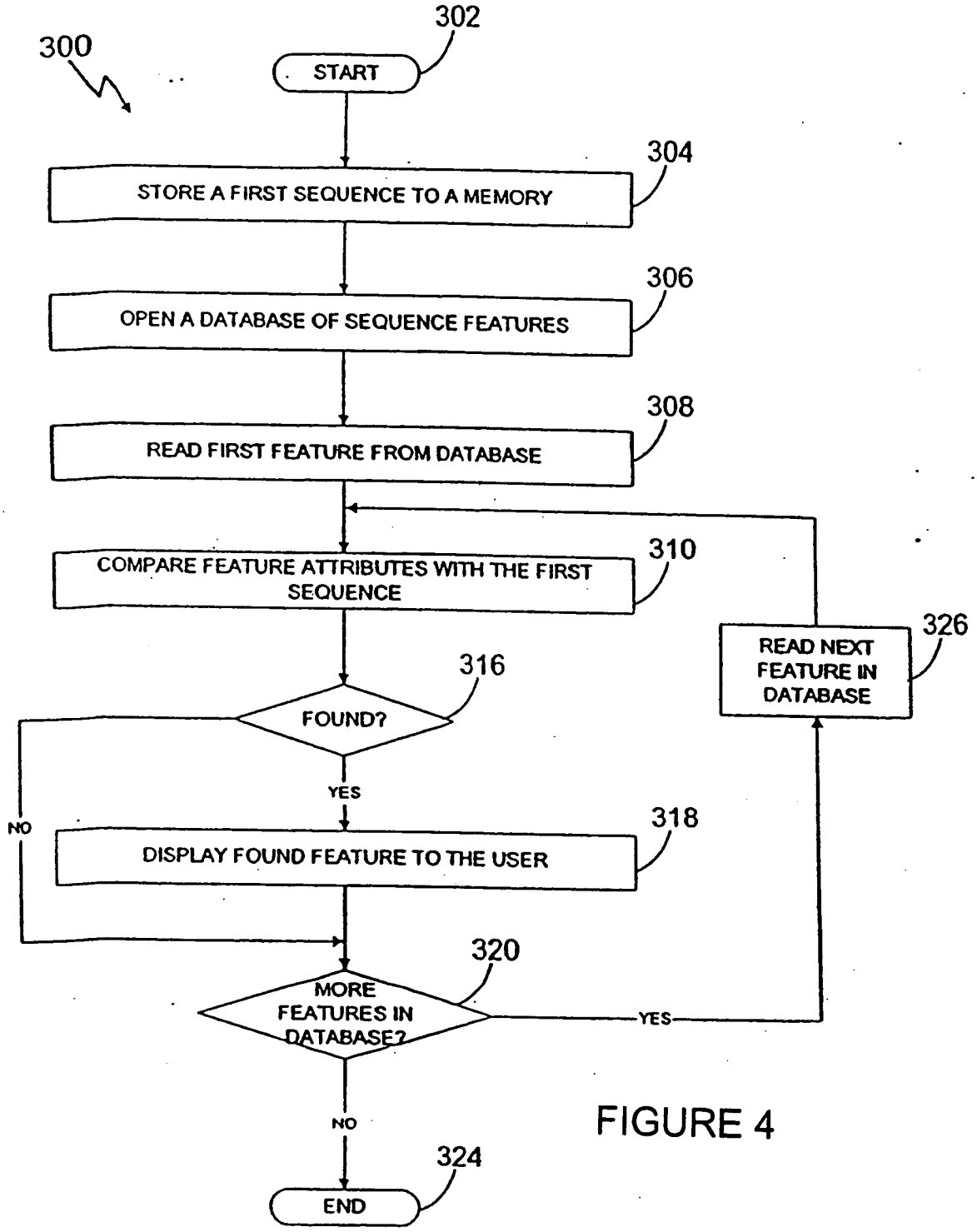

300
302
START
304
STORE A FIRST SEQUENCE TO A MEMORY
306
OPEN A DATABASE OF SEQUENCE FEATURES
308
READ FIRST FEATURE FROM DATABASE
310
COMPARE FEATURE ATTRIBUTES WITH THE FIRST SEQUENCE
316
FOUND?
YES
NO
318
DISPLAY FOUND FEATURE TO THE USER
320
MORE FEATURES IN DATABASE?
YES
NO
324
END
326
READ NEXT FEATURE IN DATABASE

FIGURE 4

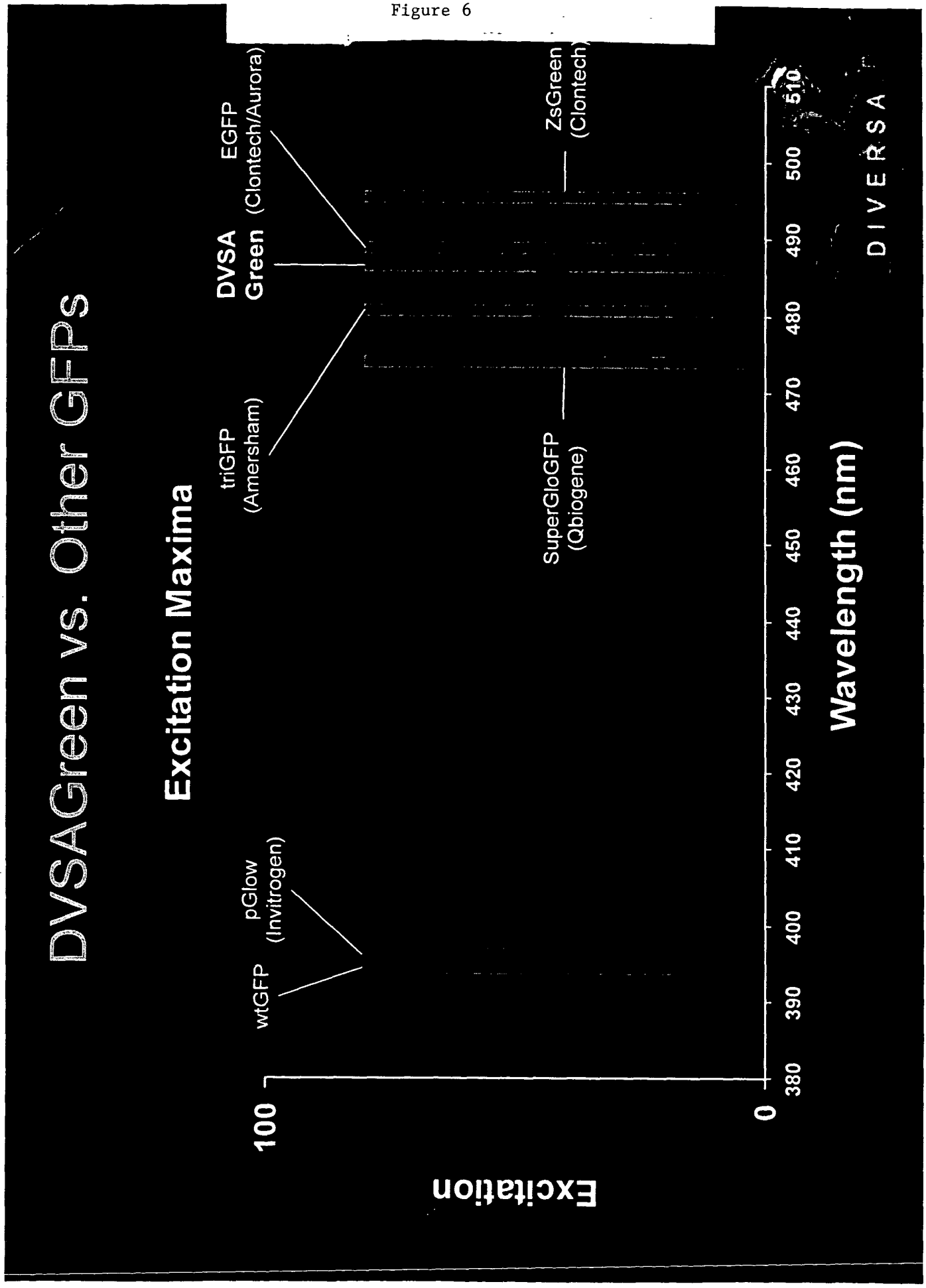
Properties of Diversa Fluorescent Proteins

	DVSACyan	DVSAGreen
Number of amino acids	227	253
Calculated subunit mass (kDa)	25.9	28.6
Total mass (kDa)	51.8	57.3
Excitation maximum (nm)	448(463)	487
Emission maximum (nm)	491	507
Quantum yield	0.76	0.61
Extinction coefficient (M^{-1} cm^{-1})	18,900	98,200

DIVERSA

Figure 5

Figure 6


DVSAGreen vs. Other GFPs
Excitation Maxima
wtGFP
pGlow (Invitrogen)
triGFP (Amersham)
DVSA Green
EGFP (Clontech/Aurora)
SuperGloGFP (Qbiogene)
ZsGreen (Clontech)
Excitation
100
0
380
390
400
410
420
430
440
450
460
470
480
490
500
510
Wavelength (nm)
DIVERSA

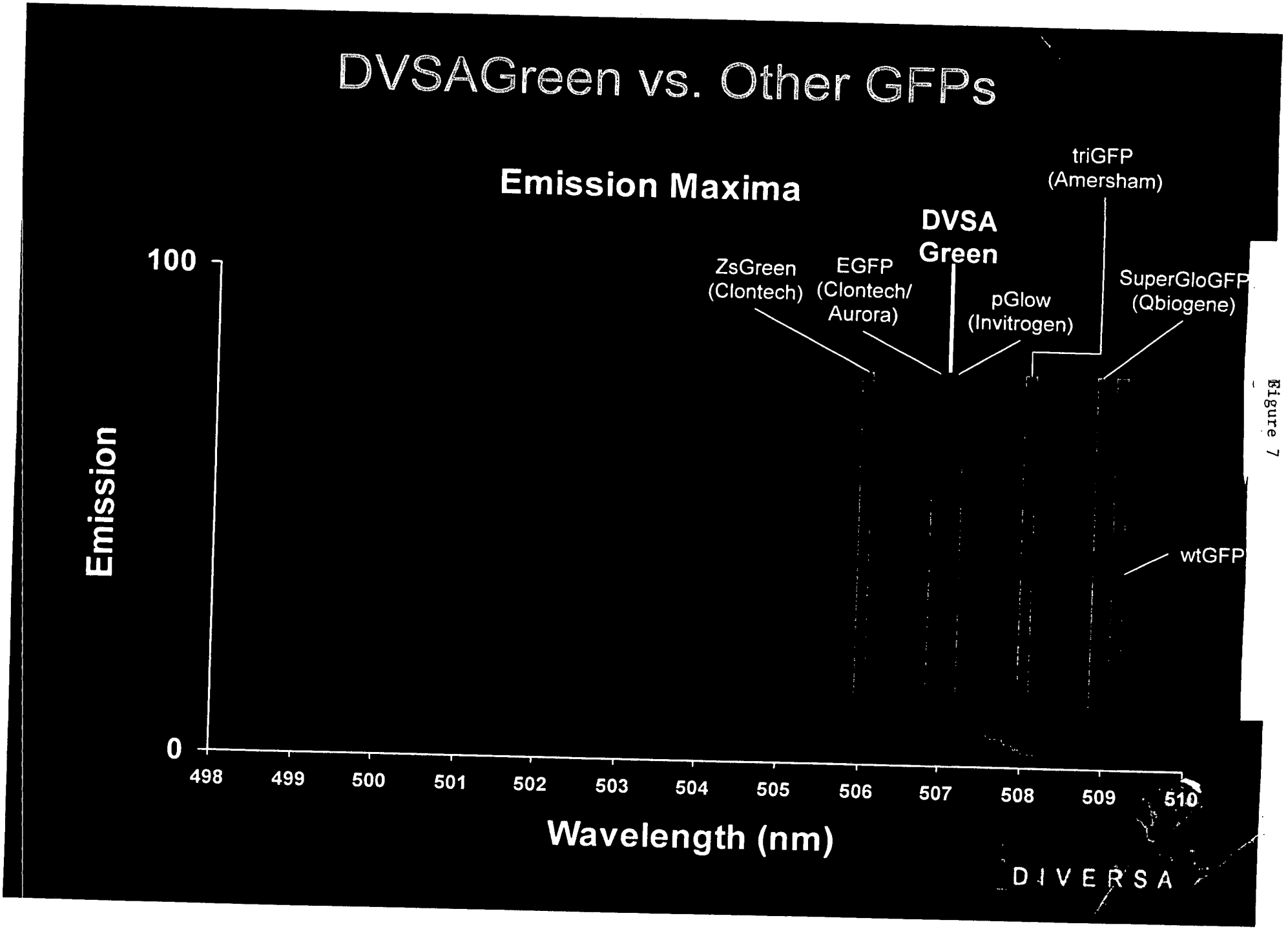

DVSAGreen vs. Other GFPs
Emission Maxima
triGFP
(Amersham)
DVSA
Green
ZsGreen
(Clontech)
EGFP
(Clontech/
Aurora)
pGlow
(Invitrogen)
SuperGloGFP
(Qbiogene)
100
Emission
wtGFP
0
498
499
500
501
502
503
504
505
506
507
508
509
510
Wavelength (nm)
DIVERSA

Figure 7

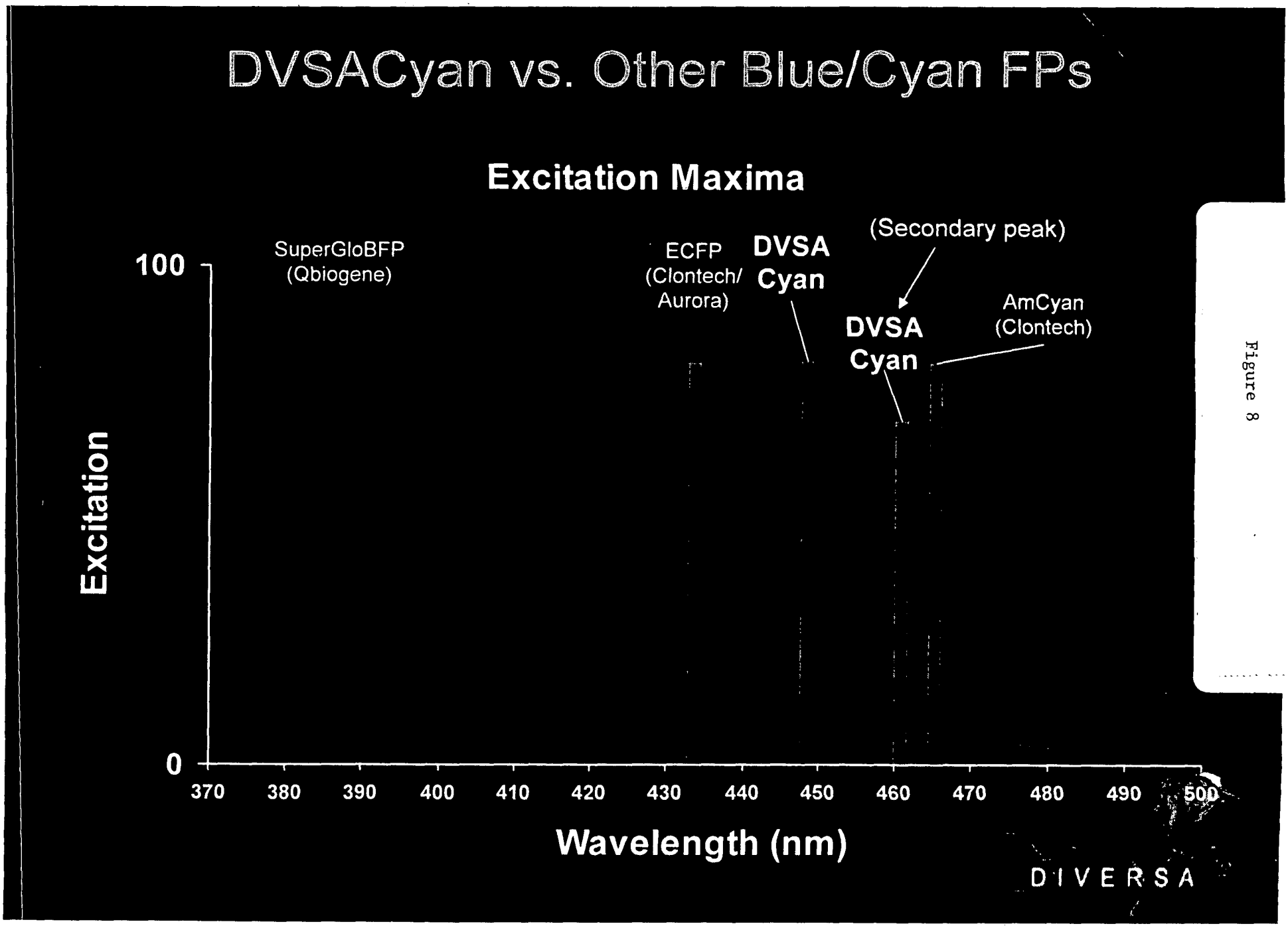

DVSACyan vs. Other Blue/Cyan FPs
Excitation Maxima
SuperGloBFP
(Qbiogene)
ECFP
(Clontech/
Aurora)
DVSA
Cyan
(Secondary peak)
DVSA
Cyan
AmCyan
(Clontech)
100
0
Excitation
370
380
390
400
410
420
430
440
450
460
470
480
490
500
Wavelength (nm)
DIVERSA

Figure 8

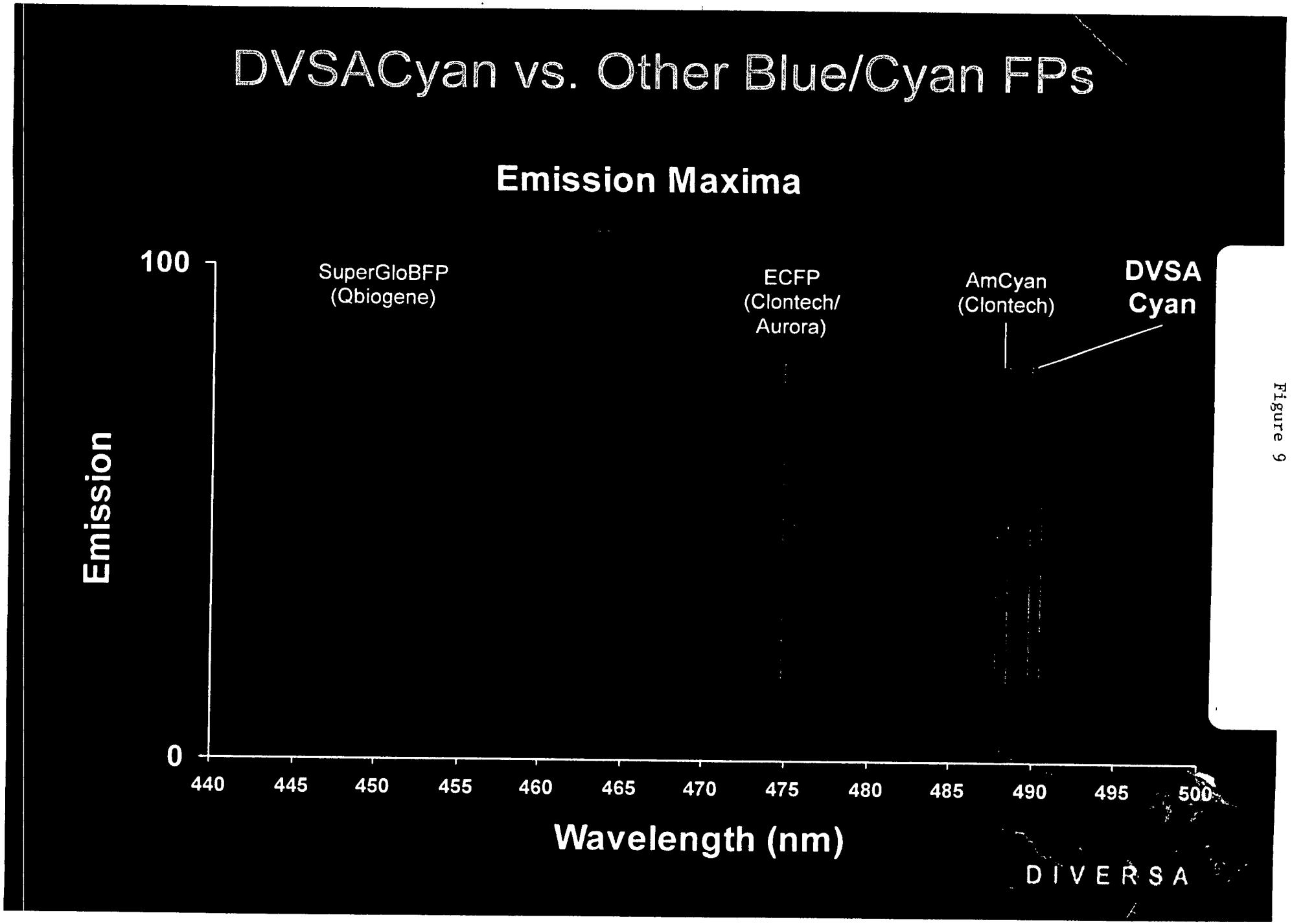
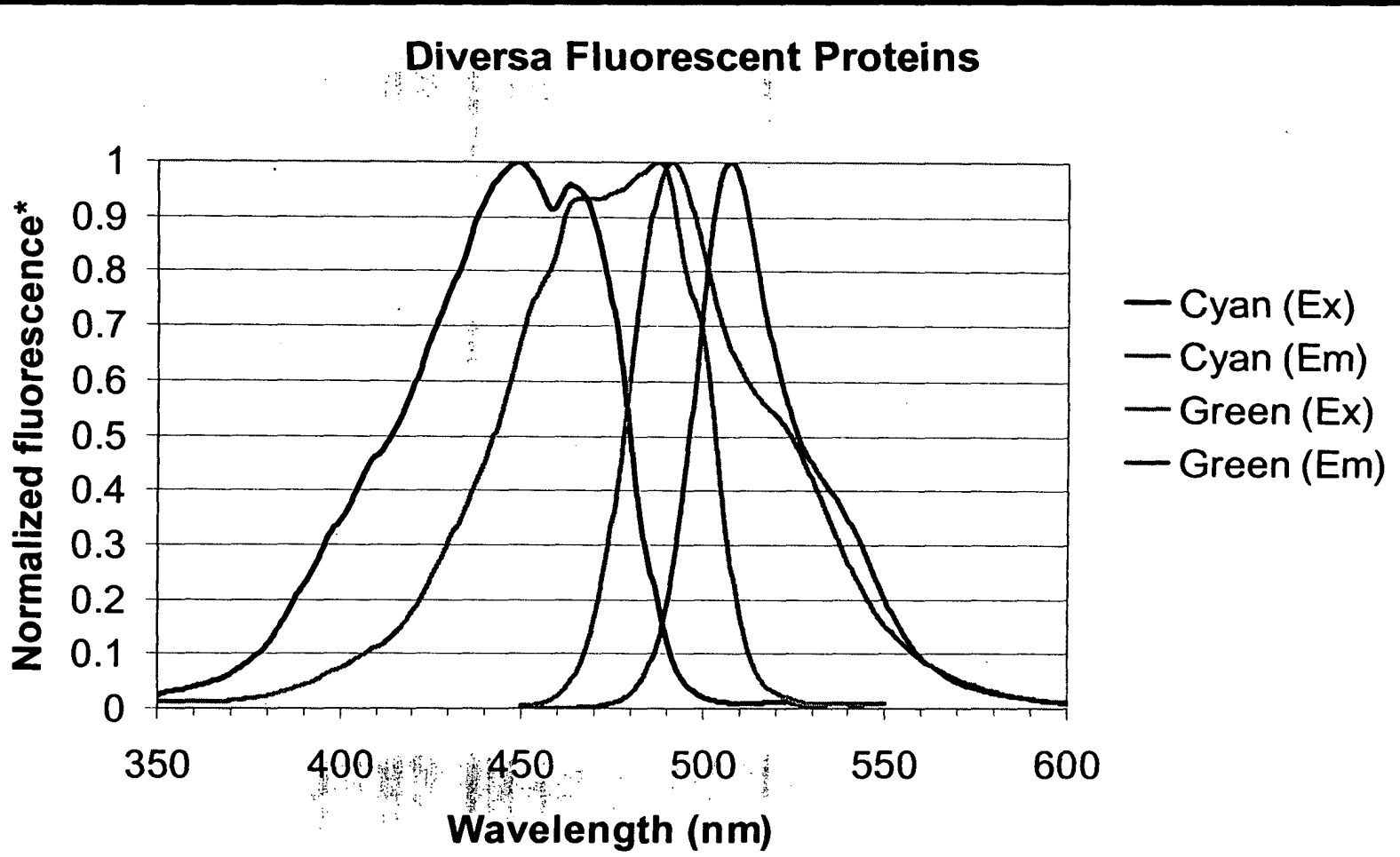

DVSACyan vs. Other Blue/Cyan FPs
Emission Maxima
100
0
Emission
SuperGloBFP
(Qbiogene)
ECFP
(Clontech/
Aurora)
AmCyan
(Clontech)
DVSA
Cyan
440
445
450
455
460
465
470
475
480
485
490
495
500
Wavelength (nm)
DIVERSA

Figure 9

Excitation and Emission Spectra



*Spectra normalized to the peak excitation and emission fluorescence for each protein

DIVERSA

Figure 10

DVSAGreen protein is brighter than EGFP

	Quantum yield	Extinction coefficient (M^{-1} cm^{-1})	Relative brightness*
wtGFP	0.77-0.80 [1,2]	21,600-27,600 [1,2]	1
EGFP	0.6-0.7 [3,4]	39,200-55,900 [3,4]	1.42-1.77
DVSAGreen	[illegible]	[illegible]	2.7-3.6
AmCyan	0.24 [2]	40,000 [2]	0.43-0.58
DVSACyan	0.76	18,900	0.65-0.88

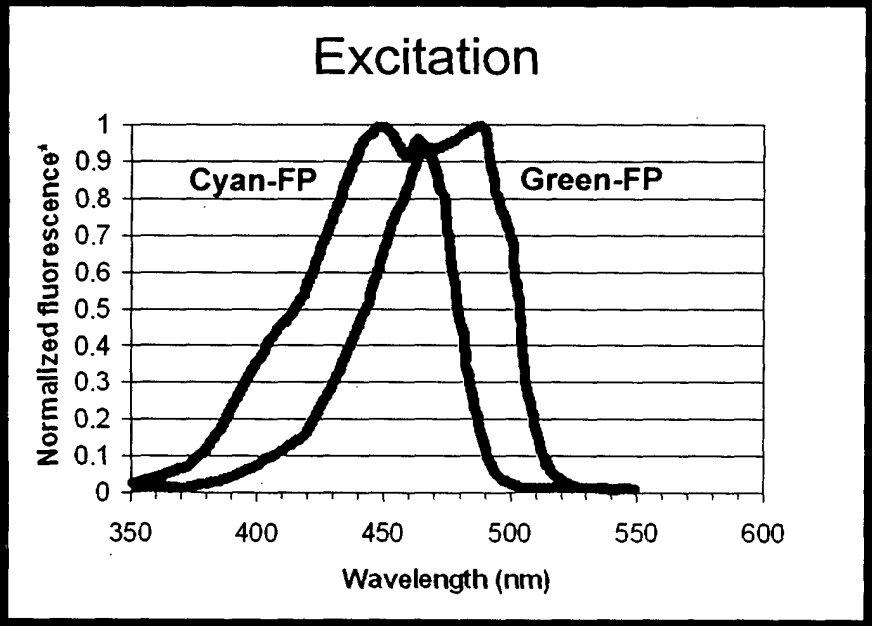
* Relative brightness (maximal extinction coefficient multiplied by quantum yield) as compared to wtGFP

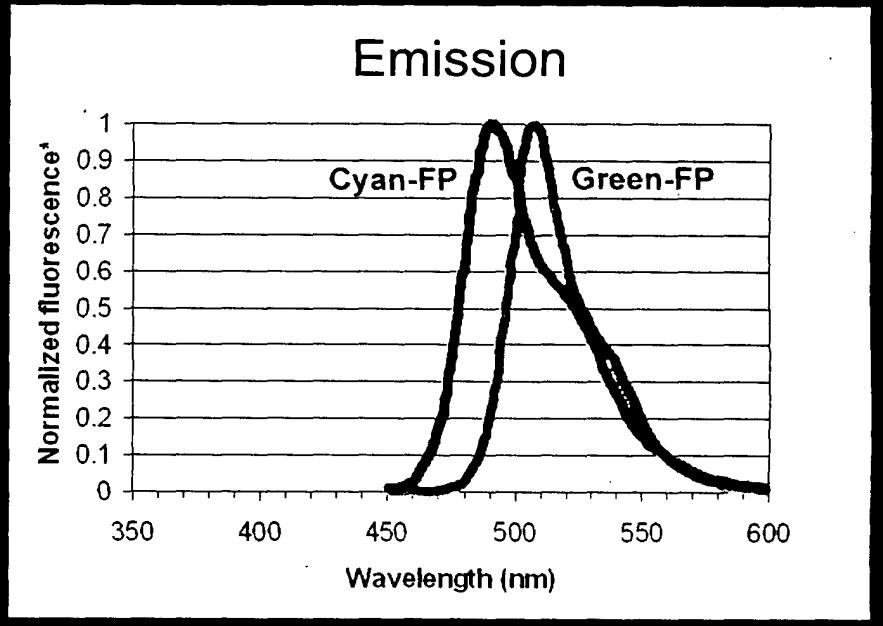
[1]Taken from Heim and Tsien, Current Biology 1996
[2]Taken from Matz et al, Nature Biotechnology, 1999
[3]Taken from Zimmer, Chemical Reviews, 2002
[4]Taken from Remington, Nature Biotechnology, 2002

DIVERSA

Figure 11

Excitation and Emission Spectra





*Spectra normalized to the peak excitation and emission fluorescence for each protein

Figure 12

DIVERSA

Relative Brightness

Comparison with Commercially Available Fluorescent Proteins

	Quantum Yield	Extinction Coefficient (M^{-1} cm^{-1})	Relative Brightness*
DiscoveryPoint™ Green FP	0.61	98,250+	2.7-3.6
Wild type AvGFP	0.77-0.80 [1,2]	21,600-27,600 [1,2]	1
EGFP	0.6-0.7 [3,4]	39,200-55,900 [3,4]	1.42-1.77
pGlow	0.79 [3]	30,000 [3]	1.1-1.4
DiscoveryPoint™ Cyan FP	0.76	18,900+	0.65-0.88
AmCyan	0.24 [2]	40,000 [2]	0.43-0.58
ECFP	0.4 [3]	32,500 [3]	0.59-0.78

*Relative brightness (maximal extinction coefficient multiplied by quantum yield) as compared to wtAvGFP, +Measured per chromophore

1. Heim and Tsien, Current Biology 1996
2. Matz et al, Nature Biotechnology, 1999
3. Zimmer, Chemical Reviews, 2002
4. Remington, Nature Biotechnology, 2002

Figure 13

DIVERSA

Summary

Summary of Diversa's DiscoveryPoint™ Fluorescent Proteins		
	DiscoveryPoint™ Green-FP	DiscoveryPoint™ Cyan-FP
Excitation/Emission max (nm)	487/507	448(463)/491
Stoke's shift (nm)	20	43(28)
Maturation time	Within 1 hour	Within 1 hour
Quantum yield	0.61	0.76
Extinction coefficient (M^{-1} cm^{-1})	98,200	18,900
Thermostable to 80°C	Yes	Yes
# of amino acids	228	227
Calculated subunit mass (kDa)	26.0	25.9
Total mass (kDa) - dimers	52.0	51.8

Figure 14

DIVERSA

SEQ ID NO: 27 Nucleotide location of segm nt (start-stop)	Overhangs on start-stop	SEQ ID NO: 29 Nucleotide location of segment (start-stop)	Overhangs on start-stop	SEQ ID NO: 31 Nucleotide location of segment (start-stop)	Overhangs on start-stop
1-53	start-GGA/CCT	1-41	start-GGA/CCT	1-43	start-CATA/GTAT
				48-92	CATA/GTAT-TCCT/AGGA
				97-142	TCCT/AGGA-GGA/CCT
57-78	GGA/CCT-TTT/AAA	45-66	GGA/CCT-TTT/AAA	146-167	GGA/CCT-TTT/AAA
82-116	TTT/AAA-AGG/TCC	70-104	TTT/AAA-AGG/TCC	171-205	TTT/AAA-AGG/TCC
120-157	AGG/TCC-CTC/GAG	108-145	AGG/TCC-CTC/GAG	209-246	AGG/TCC-CTC/GAG
161-185	CTC/GAG-ACCA/TGGT	149-173	CTC/GAG-ACCA/TGGT	250-274	CTC/GAG-ACCA/TGGT
190-224	ACCA/TGGT-CCC/GGG	178-212	ACCA/TGGT-CCC/GGG	279-313	ACCA/TGGT-CCC/GGG
228-275	CCC/GGG-CT/GA	216-266	CCC/GGG-CT/GA	317-367	CCC/GGG-CT/GA
278-323	CT/GA-AAG/TTC	269-314	CT/GA-AAG/TTC	370-415	CT/GA-AAG/TTC
327-354	AAG/TTC-TTC/AAG	318-342	AAG/TTC-TTC/AAG	419-452	AAG/TTC-TTC/AAG
358-393	TTC/AAG-CCT/GGA	346-378	TTC/AAG-CCT/GGA	456-491	TTC/AAG-CCT/GGA
397-436	CCT/GGA-CATC/GTAG	382-421	CCT/GGA-CATC/GTAG	495-534	CCT/GGA-CATC/GTAG
441-477	CATC/GTAG-GGA/CCT	426-471	CATC/GTAG-GGA/CCT	539-584	CATC/GTAG-GGA/CCT
481-500	GGA/CCT-GG/CC	475-504	GGA/CCT-GG/CC	588-617	GGA/CCT-GG/CC
503-542	GG/CC-AAG/TTC	507-546	GG/CC-AAG/TTC	620-659	GG/CC-AAG/TTC
546-593	AAG/TTC-GA/CT	550-597	AAG/TTC-GA/CT	663-710	AAG/TTC-GA/CT
596-638	GA/CT-GAG/CTC	600-639	GA/CT-GAG/CTC	713-755	GA/CT-GAG/CTC
642-end	GAG/CTC-end	643-end	GAG/CTC-end	759-end	GAG/CTC-end

Figure 15